UNIVERSITY OF GONDAR

FACULTY OF VETERINARY MEDICINE

SERO-PREVALENCE OF BRUCELLOSIS IN GOATS PURCHASED FOR SLAUGHTER IN SELECTED EXPORT ABATTOIRS, ETHIOPIA

DVM THESIS

BY

SADDAM MOHAMMED

JUNE, 2015

GONDAR, ETHIOPIA

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A thesis submitted to the Faculty of Veterinary Medicine, University of Gondar in partial fulfillment of the requirements for the degree of Doctor of Veterinary Medicine

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LIST OF ABBREVIATIONS

BBAT	Buffered Brucella Antigen Test
CDC	Center for Disease Control and Prevention
CFSPH	Center for Food Security and Public Health
CFT	Complement Fixation Test
CSA	Central Statistics Agency
FAO	Food and Agricultural Organization
ICMSF	International Commission of Microbiological Specifications for Foods
ILRI	International Livestock Research Institute
NAHDIC	National Animal Health Diagnostic and Investigation Center
OIE	Office International des Epizooties
PCR	Polymerase Chain Reaction
RBPT	Rose Bengal Plate Test
SPSS	Statistical Package for the Social Sciences
WHO	World Health Organization

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ABSTRACT

A cross sectional study was conducted from November 2014 to April 2015 to determine the sero-prevalence of brucellosis in selected sheep and goat product export abattoirs and to assess the possible association of different epidemiological risk factors with the occurrence of the disease. A total of 450 sera were collected from goats in those selected export abattoirs, using systematic random sampling technique. Rose Bengal Plate Test was used as a screening test and detected 1.56% (N=7) of the samples as sero positive. Up on further testing by CFT for confirmation, only 1.11% (N=5) of the samples were positive. In this study there was no statistically significant relationship observed between the risk factors like age, origin and sex ($P>0.05$), although higher prevalence was observed in adults (1.97%), but statistically significant relationship was observed between sero-prevalence and body condition of animals, where higher prevalence was observed in poor body conditioned goats ($p<0.05$). In conclusion even though the overall prevalence observed in this study was relatively low, the finding still has the capability to indicate the presence of the disease and the importance of intervention in the areas from which the goats are supplied or produced as there is risk of spread of the disease which is economically important. The existence of the disease in those export abattoirs may lead to prohibition of export of slaughtered goats to Middle East and other countries to preclude risk of zoonosis. This in turn results in loss of income from the export sector. Therefore, awareness creation for animal owners and implementation of strategic control measure is necessary to prevent further spread of the disease in the study area.

Keywords: *Brucellosis, CFT, Ethiopia, Export abattoirs, Goats, RBPT, Zoonoses, cross sectional study*

1. INTRODUCTION

Ethiopia is an agricultural based country and owns huge number of small ruminants, estimated to be 48.2 million head of sheep and goats (CSA, 2011). Of the total number of goats (about 21.7 million head), 70% are found in low land pastoral areas. This is because they are well adapted to hot and dry conditions and provide golden opportunity to alternatively exploit the potential of pastoral areas. Goats are highly adapted to broad range of environmental conditions. Moreover, low cost of production, requirement of little land and higher prolificacy made them attractive assets for development. This makes investment in these animals avoid losses due to high inflation rates that are found in unstable economies of many underdeveloped countries like Ethiopia. This is because sheep and goats provide rapid cash turnover (Nigatu *et al.,* 2014).

The small ruminants and their meat/milk products represent an important export commodity, which significantly contribute to the national economy. Goats together with sheep contribute to a quarter of the domestic meat consumption; about half of the domestic wool requirements; about 40% of fresh skins and 92% of the value of semi- processed skin and hide export trade. It is estimated that 1,078,000 sheep and 1,128,000 goats are used in Ethiopia for domestic consumption annually. There is also a growing export market for sheep and goats meat in the Middle Eastern Gulf states and some African countries. At optimum off take rates, Ethiopia can export 700,000 sheep and 2 million goats annually, and at the same time supply 1,078,000 sheep and 1,128,000 goats for the domestic market (Alemu and Markel, 2008).

Even though this sector contributes much to the national economy, its development is hampered by different constraints. The most important constraints to small ruminant production are poor management system, low genetic endowment, and wide spread endemic diseases. Among many factors that limit economic return from small ruminants, reproductive diseases including brucellosis are the major disease constraints (ILRI, 2006).

Brucellosis is an infectious bacterial zoonotic disease caused by member of genus *Brucella*. The disease is primary reproductive disease clinically characterized by abortion in the last trimester and retained placenta in females whereas orchitis and epididymitis with frequent sterility in males (Radostits and Inchcliff, 2000).

The incidence of the disease in humans is thus closely tied to the prevalence of the infection in sheep, goats and cattle, and practices that allow exposure of humans to potentially infected animals or their products (Kaltungo *et al.,* 2013).

Despite the presence of larger population of small ruminants in different regions of Ethiopia, very limited researches are done on brucellosis, even if it is said to be endemic in the country. The sero-prevalence of brucellosis in goat is variably reported in different parts of the country. Among this; Prevalence of 4.8% from Afar region (Ashenafi *et al.,* 2007), 1.3% from central highlands of Ethiopia (Tekeleye and Kasali, 1990), 9.7% from Afar and Somali region (Teshale *et al.,* 2007), 3.2% from Southern Ethiopia region (Mengistu *et al.,* 2007), 2.12% in some export abattoirs were reported (Nigatu *et al.,* 2014). Occurrence of such disease might result in loss of income from prohibition of export of live animals and their products due to its public health significance. Therefore, it was found important to study brucellosis in goats purchased for slaughter in those selected export abattoirs with the objective of:

- Determining the sero-prevalence of brucellosis in goats in those selected export abattoirs
- Assessing some of the possible epidemiological risk factors that might contribute for the occurrence of brucellosis in goats.

2. LITERATURE REVIEW

Brucellosis is an infectious bacterial disease caused by microorganisms of the genus *Brucella* and affecting a number of animal species (yesuf *et al.,* 2010).

2.1. Etiology

In goats brucellosis is mainly caused by *Brucella melitensis*, a gram negative, facultative intracellular pathogen. *B. melitensis* contains three biovars (biovars 1, 2 and 3). All the three biovars cause the disease in goats, but their geographic distribution varies. *Brucella abortus* and *Brucella suis* infection also occur occasionally in goats, but clinical disease seems to be rare (CFSPH, 2009).

2.2 Culture, Morphology and Staining Characteristics

Brucella members are aerobic, but some strains require 5-10% carbon dioxide (CO2) added for growth, especially on primary isolation. The optimum pH for growth varies from 6.6 to 7.4 and culture media should be adequately buffered near pH 6.8 for optimum growth. The optimum growth temperature is 36-38°C, but most strains can grow between 20°C and 40°C. *Brucella* requires biotin, thiamin and nicotinamide. The growth of most *Brucella* strains is inhibited on media containing bile salts, tellurite or selenite (European commission, 2001).

Brucella will appear as punctuate colonies after 48 h of incubation. Colonies are non pigmented and non hemolytic. They have a characteristic Gram stain morphology that is extremely helpful in differentiating them from other gram negative organisms. *Brucella* cells appear as tiny, faintly stained cocco-bacilli (CDC, 2001)

Brucella species may also appear as short rods, 0.6 – 1.5 µm long by 0.5 – 0.7 µm width. They are arranged singly and less frequently in pairs, short chains or small groups. It doesn't possess capsule, spore and flagella. *Brucella* spp. are Gram-negative and usually do not show bipolar staining. They are not truly acid-fast but resist decoloration by weak acids, thus stain red by the Stamp's modification of Ziehl-Neelsen method (European commission, 2001).

2.3 Epidemiology

Brucellosis is one of the most wide spread zoonotic disease globally. *Brucella* spp. are capable of causing disease in a variety of animal species, including humans (Garrity *et al*., 2001).

Table 1**.** Zoonotic potential and host preferences of Brucella species

Species	**Zoonotic potential**	**Host preference**
Brucella melitensis	High	Goat, Sheep
Brucella abortus	Moderate	Cattle
Brucella suis	Moderate	Pig
Brucella canis	Mild	Dog
Brucella ovis	Absent	Sheep
Brucella neotomae	Absent	Desert wood rat *(Neotomae lepidae)*
Brucella ceti	Mild	Cetaceans
Brucella pinnipedialis	Mild	Seals
Brucella microti	Absent	Common voles (*microtus arvalis)*

Source: Xavier *et al* (2010)

Brucella melitensis is the most virulent species of *Brucella* genus and has three biovars, with biovar 1 and 3 being the ones isolated frequently in small ruminants in the Mediterranean, the Middle East and Latin America (Benkirane, 2006).

Goats are the classic and natural hosts of *B.melitensis* and, together with sheep, are its preferred host. In pathological and epidemiological terms, *B.melitensis* infection in goats is similar to *B.abortus* infection in cattle (Aparicio, 2013).

2.3.1 Geographical Distribution

Of the three different biovars of *B.melitensis* biovar 3 predominates almost exclusively in Mediterranean countries and Middle East, while biovar 1 seems to predominate in Latin America. The biovars 1 and 2 have also been reported in some southern European countries. In the European Union the following Member States and regions have been recognized as being free from *B.melitensis*: Belgium, Denmark, Finland, Germany, Ireland, Luxembourg, the Netherlands, Sweden, the United Kingdom, 17 Departments of France and 2 Provinces of Spain (European commission, 2001).

Distribution of *B. melitenesis* in some countries of Africa is indicated in the following table.

Table 2. Distribution of *B.melitenesis* in some countries of Africa.

Country	*B.melitenesis*
Egypt	+
Ethiopia	+
Kenya	+
Sudan	+
Somalia	+
Eritrea	+
Libya	ND
Algeria	+
Tunisia	ND
Niger	++
CoteD'ivory	+
Zimbabwe	-
Botswana	+
Nigeria	-
South Africa	+

Remark: ++ = *high prevalence*, + = *sporadic low prevalence*, ND = *no data*

Source: FAO, (2003).

Ethiopia

There is paucity of data on the status of data on small ruminant brucellosis in Ethiopia. The following table shows the reports of different research on brucellosis in goats in different parts of Ethiopia.

Table 3. Prevalence of goat brucellosis in Ethiopia

Reporters	**Reported prevalence**	**Areas of Reports**
Tekelye and Kasali (1990)	1.3%	Central highlands
Yibeltal (2005)	16%	Afar region
Teshale *et al*. (2006)	1.7%	Somali region
Ashenafi *et al.* (2007)	5.8%	Pastoral region of Afar
Mengistu (2007)	2.0%	Southern Ethiopia
Bekele (2011)	2.0%	Jijiga
Nigatu *et al.* (2011)	2.12%	Selected export abattoirs
Dabassa *et al.* (2013)	1.88%	Yabello district
Tsehay *et al*. (2014)	3.09%	pastoral areas of Somali and Oromia region

2.3.2 Age and Sex

B.melitensis infection causes disease only in adult (sexually mature) animals. Young animals may be infected but do not show any clinical sign and generally show only a weak and transient serological response. However, susceptibility increases after sexual maturity and especially with pregnancy (European commission, 2001). In *B. melitenesis* infection, male goats are less susceptible than females (FAO, 2003). According to the study of Ashenafi *et al.* (2007), prevalence rate of 5.3% was observed in adult and 1.6% in younger goats. While Nigatu *et al.* (2014) and Tsehay *et al.* (2014) observed no statistically significant difference in susceptibility between various age groups.

2.3.3 Breed

Exotic breeds and their hybrids are found to be at higher risk; this may be associated with their better productivity and intensive management (Garrido *et al.*, 2001).

2.3.4 Transmission

The main route of infection is via suckling or licking of aborted fetus and their placentas as well as vaginal discharges. The infection may also be transmitted from goats to kids—a small percentage in utero, but the majority via the ingestion of infected milk or colostrums. These kids greatly increase the difficulty of eradicating *B.melitensis* from the herd or flock as the infection may persist until adulthood in these kids without the induction of detectable antibodies (Coetzer and Tustin, 2004). The organism can survive on pasture for several months but transmission by fomites is believed to have no practical significance (Radostitis *et al.*, 2007).

2.4 Pathogenesis

The mucosal surface of the alimentary tract is the principal route of entry for *B. melitensis* (Carlos *et al*., 2011). Unlike many other pathogens, *Brucella* spp. invade host cells without activating innate immune defence systems and then resist intracellular killing to persist in the host (Gorvel and Moreno, 2002; Barquero *et al*., 2007).

The major virulence mechanisms of Brucella already identified are those required for host cell invasion and intracellular survival or replication (Arellano, 2005).

Brucella spp. are capable of invading and surviving in both phagocytic and non phagocytic host cells (Celli, 2006). Macrophages, dendritic cells (DCs), and trophoblasts represent the major target cells for *Brucella*. In order to reach its target cells, *Brucella* needs to cross mucosal barriers of respiratory, genitourinary or digestive tract, where it undergoes phagocytosis by resisdent macrophages and DCs, resulting in dissemination of the organism to lymphoid and reproductive organs (Xavier, 2010).

Once ingested, some of *Brucella* organisms are able to evade or hinder the phagolysosomal action of the neutrophils and macrophages by redirecting the intracellular trafficking of phagolysosomal action (Dornand *et al*., 2002; Gorvel and Moreno, 2002; Franco *et al.*, 2007). The virulence factor VirB, is a secretory pump that selectively pumps proteins and macromolecules and is critical in the pathogenesis and virulence of Brucellosis infection (Franco *et al.*, 2007).

The persistence of the bacteria in the phagocytic cells allows for bacterial replication in these cells. Replication leads to release of the bacteria from the cells, thus resulting in bacteremic phase. This bacteremia allows for colonization of the bacteria in multiple tissues, but in livestock the bacteria most frequently colonize in the lymphoid tissues, mammary gland and reproductive tract (Ragan, 2002).

The localization of *Brucella* spp. in reproductive tract leads to colonization of the chorionic trophoblast of the placenta in pregnant livestock (Corbel, 2006). The resulting placentitis caused by replicating bacteria results in ulceration of the chorioallantoic membrane while sparing the endometrium of uterus. The resulting pathology leads to late gestation abortions in naively infected

livestock (Radostits *et al.,* 2010). The presence of erythritol in the testes of male species leads to colonization of Brucella spp. in their reproductive tracts with a resulting epididymitis and orchitis (Enright, 1990).

2.5 Clinical Signs

According to Coetzer and Tustin (2004) clinical signs associated with B. melitensis infection in goats include: abortion storm in susceptible herd usually in late gestation, kids from infected females may be born weak or asymptomatic, retention of placenta and fetal membranes especially in nany goats, copious genital secretions which may persist for up to 3 months following abortion, decreased milk yield as well as quality, orchitis or epididymitis and hygroma.

2.6 Diagnostic Methods

Diagnostic tests can be applied with different goals: confirmatory diagnosis, screening or prevalence studies, certification, and in countries where brucellosis is eradicated, surveillance in order to avoid re introduction of the disease through importation of infected animals or animal products. these methods include direct tests, involving microbiological analysis or DNA detection by polymerase chain reaction (PCR)-based methods and indirect tests, which are applied either in vitro (mainly to milk and blood) or in vivo (allergic tests) (Godfroid *et al.,* 2010).

The most widely used serum-testing procedures for the diagnosis of *Brucella* infections in goats are the buffered *Brucella* antigen tests (BBAT), and the complement fixation test (CFT) which are considered by the OIE also as " prescribed tests for trade" (OIE, 2009).

The World Organization for Animal Health (OIE) prescribes the use of a buffered Brucella antigen test called the buffered plate antigen test and the Rose Bengal Test (RBT) as approved screening tests and the complement fixation test as the confirmatory test (Havas, 2011).

2.7 Treatment

Treatment is unlikely to be undertaken in animals and is also unlikely to be economically or therapeutically effective. A cure rate of 65% and 100% respectively was reported intra peritoneal

administration of 500mg and1000mg of tetracycline to naturally infected goats for a period of 6 weeks. A dose of 100mg of long acting tetracycline given every 3 days for a period 6 weeks achieved a cure rate of 75% (Radostitis *et al.,* 2007).

2.8. Control and Prevention

Reducing brucellosis zoonosis requires reducing the exposure of humans to the disease agent. There are two primary exposure patterns of importance that can be targeted for intervention. The first is occupational-associated exposure. The second route of exposure is through contaminated food such as fresh and soft cheese made from raw milk, and other dairy products made from raw milk with a high water content, low salinity, and low curing time in high salinity and a pH > 5 (Corbel, 2006).

Occupational disease is controlled through the animal population. There is no human vaccine available to directly protect the workers (Shurig *et al.,* 2002), but there are effective *B. melitensis* vaccines for small ruminants (Radostits *et al.,* 2007). The small ruminant *B. melitensis* vaccine is the Rev 1 modified live vaccine; hence, vaccination in pregnant animals cause abortion, interfere with serologic tests, complicating disease control and eradication programs (Havas *et al.,* 2011).

Mass vaccination of livestock together with other measures such as movement control and testing and isolation of infected animals, can effectively control brucellosis in the animal population and eventually reduce the transmission to the human population (Smits and Kadri, 2005).

Direct food safety techniques can be applied to dairy products. From all meats, except those contaminated with *B. suis,* the risk of infection via consumption is rare (ICMSF, 1996). The majority of risk comes from the contamination of dairy products. *Brucella* spp. are readily killed by pasteurization or heating of raw milk (Havas *et al.,* 2011).

According to Yohannes *et al.,* (2013), in areas where the disease is less prevalent (for example, livestock sero-prevalence of less than 1%), it is recommended to practice test and cull policy with compensation. For areas with high and moderate prevalence (>5%) under well-organized farming systems, it is recommended to practice test and segregation policy by which animals with brucellosis will be isolated and products consumed after pasteurization, with animals being disposed properly at the end of their productive live.

There are many component parts to eradicate the disease i.e. test and slaughter, continued vaccination of replacement stock, animal movement control, availability of disease-free replacements of the slaughtered animals and an adequate surveillance system (Corbel, 2006).

2.9 Public Health Aspect

Brucellosis is one of the world's major zoonoses, that still is of veterinarian, public health and economic concern in many parts of the world (Smits and Kadri 2005). Brucellosis was first recognized as a disease affecting humans on the Island of Malta in the early 20th century. Though its distribution is worldwide; yet brucellosis is more common in countries with poorly standardized animal and public health program (Capasso, 2002).

Animals are the only significant source of human brucellosis, and transmission is via direct contact (e.g. veterinarians, abattoir workers, lab workers, and livestock keepers) and through consumption of unpasteurised dairy products. Human brucellosis is a grave and debilitating disease that may lead to permanent sequelae, requires prolonged and combined antibiotherapy, and is fatal in 1%–5% of untreated cases (Ducrotoy *et al.,* 2014). In humans, brucellosis can be caused by *B.abortus*, *B.melitensis*, *B.suis* biovar 1-4 and, rarely, *B.canis* or marine mammal *Brucella*, but *B.ovis*, *B. neotomae*, and *B.suis* biovar 5 have not been associated with human disease (Lopes *et al.,* 2010). Of this, *B.melitensis* is highly pathogenic and considered to be the most sever human pathogen in the genus (CFSPH, 2009).

Humans are incidental host of brucellosis, and the pathogenesis from initial infection to phagocytic cell uptake is identical to animal hosts. The lysis of phagocytic cells that releases the *Brucella* organisms also results in release of cellular debris and pyrogenic endotoxins that cause an episode of fever when this occurred repeatedly in different infected phagocytic cells, so can the fever-undulant fever (Havas *et al.,* 2011). After an incubation period of 8 to 20 days symptoms include lassitude, headache and muscular or joint pain, and drenching sweats, especially at night (European commission, 2001).

The epidemiology of human brucellosis has drastically changed over the past few years because of various sanitary socio-economic and politic reasons, together with increased international travel.

New foci of brucellosis have emerged, particularly in central Asia, while the situation in certain countries of the Middle East is rapidly worsening. The disease occurred worldwide except in those countries where animal brucellosis has been eradicated (Pappas *et al.,* 2006b).

As compared to animal brucellosis, study of human brucellosis in Ethiopia is sparse. For instance out of 56 cases with fever of unknown origin, two (3.6%) were reported to be positive for *B.abortus* antibody by RBPT and CFT (Tolosa *et al.,* 2007).

A study conducted in traditional pastoral communities by Ragassa *et al.* (2009) using *B.abortus* antigen revealed that 34.1% patients with febrile illness from Borena, 29.4% patients from Hamer and 3 % patients from metema areas were tested positive. Studies conducted in high risk group such as farmers, veterinary professional, meat inspectors and artificial insemination technicians in Amhara regional state (Mussie *et al.,* 2007b), sidama zone of Southern nations and nationalities state (Kassahun *et al.,* 2007) and Addis Ababa (Kassahun *et al.,* 2006) found a sero-prevalence of 5.3%, 3.78% and 4.8% by screening sera from 238, 38 and 336 individuals respectively. The discrepancy between Ragassa *et al.* (2009) and others might be due to difference in milk consumption habits and sensitivity of the test methods used.

2.10 Economic Importance

Brucellosis is consistently ranked among the most economically important zoonoses globally (Perry and Grace, 2009). In low income countries, brucellosis is endemic and neglected, with large disease and livelihood burdens in animals and people and almost no effective control (McDermott and Arimi, 2002). The economic losses stem from reduction in meat yield, abortion, still birth, repeat breeding, banning of export of live animals and their products and infertility problems (Radostits, *et al.,* 2010).

Table 4: Costs to be considered and estimated in planning brucellosis control and eradication programs

Actors		**Cost of illness**	**Cost of prevention**
Private	Individuals and households	Treatment, loss of house hold production	Risk mitigation such as boiling milk
	Livestock sectors	Treatment, herd slaughter, market loss due to risk of infected meat & milk, mortality, morbidity, lower production, loss of exports	Increased biosecurity, vaccination and procedures to control disease along the value chain (pasteurization)
Public	Health sector (human and animal)	Treatment(hospital provision, etc), outbreak costs, movement restrictions, culling, vaccination	Risk mitigation such as movement control and vaccination, disease surveillance research
	Economy	Indirect effects on economic development, ecosystem services and tourism	Biosecurity, avoiding wild life and vectors, disease surveillance, research

Source: McDermott *et al.* (2013).

3. MATERIALS AND METHODS

3.1 Study Area and Abattoirs

The study was conducted in some selected export abattoirs found in Debre-Zeit and Modjo town. Debre-Zeit is located between latitude of 8^{o} 45′ N and longitude of 38^{o} 59′ E and it is 47.9 km South East of Addis Ababa, the capital city of Ethiopia, While Modjo town is the center of Lume district in Eastern Shewa Administrative Zone of Oromia Regional State. It is located 70 kilometers south east of Addis Ababa 8^{o}N and 39^{o}E at an altitude of 1777 meter above sea level (CSA, 2013).

The animals were supplied from different parts of the country. For most of the areas the average annual rainfall range from 400 to700 mm and with the mean daily temperature is 25-44^{o}C, while for few others rain fall pattern can be characterized as erratic, unreliable and unpredictable with average rain fall of 200 mm. average daily temperature of 28-44^{o}C (CSA, 2011).

The slaughtering capacity of these export abattoirs range from 800 up to 1500 goats per day on average and they export mutton, goat meat and edible organs like liver, kidney and heart to the Middle East countries.

3.2 Study Population

About 450 sera were collected from goats that were purchased for slaughter. All the study animals were male and unvaccinated against *Brucella*. The origins of the animals were different areas in the country. The animals were purchased from farmers on weight basis and certain weight ranges were approved depending on the customers' preferences. Mostly those goats weighting 10-12kg were rejected. The study animals were with different age category mainly within a range of 1-4 years. Their age was determined based on their dental eruption patterns. In general according to Steel (1996) goats were classified as young and adult i.e., young if 1-3 years old and having up to four permanent teeth and adult if 4-5 years and greater than four permanent teeth (Annex-1and 5).

3.3 Sampling Method and Sample Size

The sampling method employed was systematic random sampling by choosing the first case and the interval between cases with lottery method. The sample size was determined according to Thrusfield (2005) as indicated below. Previous study conducted by Nigatu *et al.,* (2014) on the prevalence of brucellosis in goats in the same area showed 2.12%. Therefore, using 2.12% as expected prevalence and 5% absolute precision at 95% confidence level, the number of animals needed in the study was 32. However, to increase the level of precision of the prevalence, the sample size was increased to more than 10 folds i.e. 450.

$$n = 1.96^2 \frac{Pexp\ (1-Pexp)}{d^2}$$

where: n = sample size

Pexp = expected prevalence

d = desired absolute precision

3.4. Data Recording

While collecting the blood specimens from study animals, we recorded the data corresponding to each animal such as origin, body condition, species, sex, and age in pre designed recording sheet (Annex-2).

3.5 Blood Sample Collection

About 10ml of blood was collected from the jugular vein of each goat using sterile plain vacutainer tubes and needles. Each sample was labeled using codes describing the specific animal. Blood was allowed to clot for 1-2 hours at room temperature, stored in slant position overnight at 4^{o}C then serum was separated from clotted blood. Separated serum was collected in a screw caped sterilized plastic vial and was stored at -20 ^{o}C until tested.

3.6. Laboratory techniques

3.6.1 Rose Bengal Plate Test (RBPT)

Rose Bengal Plate Test (RBPT) was used as a screening test for presence of *Brucella* antibody in the serum samples collected. According to the procedure described by OIE, (2009) the antigen used was Rose Bengal antigen, which constitutes a suspension of *B. abortus* inactivated by heat and phenol dispensed in an acidified buffered and stained by Rose Bengal. The test was carried out at the National Animal Health Diagnostic and Investigation Center (NAHDIC), Sebeta, Ethiopia which is certified for ISO/IEC 1705/25 (Annnex-3).

3.6.2 Complement Fixation Test (CFT)

All the RBPT positive sera were re-tested using CFT (also carried out at NAHDIC) for further confirmation according to the protocol described in OIE manual (2009). Standard *B. abortus* antigen (0.2 CH 63) which was supplied from AHVLA was used for CFT to confirm the presence of anti-*Brucella* antibodies in the sera (Annex- 4).

3.7. Data Management and Analysis

Data collected from abattoirs and obtained in laboratory were entered into a computer, on Microsoft Excel spread sheet. Statistical analysis (descriptive analysis) was performed using 'Statistical Package for the Social Sciences' (SPSS) version 16. The degree of association between each risk factor was assessed using the Chi-square (x^2) test. For all analyses, a *p*-value of less than 0.05 was taken as significant.

4. RESULTS

A total of 450 sera were tested for the presence of serum antibodies against *Brucella* infection in goats in those selected export abattoirs. Seven sera were found positive up on RBPT; up on re-testing of these samples for confirmation using CFT, only five sera were found to be positive.

Table 5**:** Sero -prevalence of brucellosis in goats in relation to Abattoirs in selected export abattoirs, Ethiopia

Abattoirs	**Number of Samples tested**	**RBPT**		**CFT**	
		Negative	**Positive**	**Negative**	**Positive**
Abt-1	50	49	1(2%)	49	1(2%)
Abt-2	50	50	0(0%)	50	0(0%)
Abt-3	100	98	2(2%)	99	1(1%)
Abt-4	150	147	3(2%)	148	2(1.3%)
Abt-5	100	99	1(1%)	99	1(1%)
Total	**450**	**443(98.4%)**	**7(1.56%)**	**445(98.9%)**	**5(1.1%)**
		χ^2 (4) =1.379, P=0.848		χ^2 (4) = 1.011, P=0.908	

Remark: - *"Abt 1-5"= code for abattoir 1 to 5*

Sero-prevalence of 2% (1 out of 50), 0% (0 out of 50), 1% (1 out of 100), 1.3% (2 out of 150), and 1% (1 out of 100) was recorded in Abattoir 1, 2, 3, 4, and 5 respectively. The difference was not statistically significant ($p>0.05$) (Table-4).

Sero-prevalence of 0.4% (1 out of 247) and 1.97% (4 out of 203) was observed in young and adult goats, respectively and the difference in prevalence was not statistically significant (Table 5).

Table 6**:** Sero -prevalence of Brucellosis in goats in relation to Age in selected export abattoirs, Ethiopia

Age category	**Number of animals**	**RBPT**		**CFT positive**	
		Negative	Positive	Negative	Positive
Young	247	245	2(0.8%)	246	1(0.4%)
Adult	203	198	5(2.46%)	199	4(1.97%)
Total	**450**	**443**	**7**(1.56%)	**445**	**5**(1.11%)
		χ^2 (1) = 1.989, P=0.158		χ^2 (1) = 2.486, P =0.115	
		OR (adult) = 3.093		OR(adult) = 4.945	

Table 7**:** Sero-prevalence of brucellosis in goats in relation to body condition in selected Export abattoirs, Ethiopia

Body condition	**Number of animals**	**RBPT**		**CFT**	
		Negative	**Positive**	**Negative**	**Positive**
Poor	31	25	6(19.35%)	26	5(16.1%)
Medium	118	117	1(0.8%)	118	0 (0%)
Good	301	301	0(0%)	301	0 (0%)
Total	**450**	**443**	**7(1.56%)**	**445**	**5(1.11%)**
		χ^2 (2) = 69.27, P = 0.00		χ^2 (2) = 68.34, P = 0.00	

Sero- prevalence of 16.1% (5 out of 31), 0% (0 of 118) and 0% (0 out of 301) were observed, in poor, medium, and good body conditioned goats respectively. The difference in prevalence is statistically highly significant ($p<0.05$) (Table 6).

Table 8**:** Sero-prevalence of brucellosis in relation to origin of animal in selected export abattoirs, Ethiopia

Origin	**Number of animals**	**RBPT**		**CFT**	
		Negative	Positive	Negative	Positive
Boran	122	119	3(2.45%)	120	2(1.63%)
Arbaminch	104	101	3(2.88%)	101	3(2.88%)
Somali	111	110	1(0.90%)	111	0 (0%)
Harar	113	113	0(0%)	113	0 (0%)
Total	**450**	**447**	**7(1.56%)**	**445**	**5(1.1%)**
		χ^2 (3) = 3.94, P = 0.267		x^2 (3) =5.804, P=0.122	

Sero-prevalence of 1.63% (2 out of 122), 2.9% (3 out of 104), 0% (0 out of 111), and 0% (0 out of 113) were recorded in Boran, Arbaminch, Somali, and Harar respectively. The difference in prevalence was not statistically significant ($p > 0.05$) (Table 7).

5. DISCUSSION

The present study indicated that, the overall sero-prevalence of brucellosis in goats in those selected export abattoirs to be 1.56% (N=7) with RBPT and 1.11% (N=5) with CFT. Two of the samples tested positive for brucella antibodies by RBPT, tested negative by CFT. This could be due to cross-reactions between *Brucella* and other bacteria which share similar epitopes, which might result in false positive result.

A 1.11% CFT confirmed finding of this study was in line with previous studies conducted by Tekelye and Kasali (1990), who reported a sero-prevalence of 1.3% in goats from central highlands, Bamaiyi *et al.,* (2015), who reported sero-prevalence of 0.91% in goats from Malaysia, and Ferede *et al.,* (2011) who reported a sero-prevalence of 0.87% in goat from Bahir Dar.

The sero-prevalence of Brucellosis in goats in this study was lower than most of the sero-prevalence reported by different previous studies i.e. lower than studies by: Yibeltal (2005), who documented a prevalence of 16% in Afar region, Mengistu *et al*. (2007) who reported 3.2 % prevalence in goats in southern Ethiopia, Ashenafi *et al.* (2007) who reported a sero-prevalence of 5.8% in goats of pastoral regions of Afar, Bekele (2011) who reported 2 % sero-prevalence in goats in Jijiga, Dabassa *et al*. (2013) who documented 1.88 % in goats in Yabello district, and Nigatu *et al*. (2014) who reported 2.12 % sero-prevalence, and Tsehay *et al*. (2014) who reported 3.09 % sero-prevalence in goats of pastoral areas of Somali and Oromia region.

The difference in the sero-prevalence of brucellosis between the current and previous studies might be due to difference in geographical location of sampled animals since abattoirs purchased both from highlands and lowlands of various regions, sample size, and/or the test protocols used in the study.

A 1.11 % CFT confirmed prevalence of this study appeared generally to be low when compared with most previous studies involving both female and male animals. This might be because of infected male animals are usually observed to be non reactors to serological tests due to low antibody titer (Crawford *et al.,* 1990), and because serological tests under estimate brucellosis in males due to the colonization of the bacteria in the testes and reticulo-endothelial system (Lapriak, 1982).

Since all study animals were caprine, male (due to unslaughter of female animals in those export abattoirs) and local breeds, no statistics had been computed on species, sex and breed. However, comparison of sero-prevalence of caprine brucellosis was carried out for different epidemiological risk factors like origin, age and body condition of goats. Accordingly, there were no statistically significant variations observed between brucellosis sero-prevalence and origin of the animals ($P>0.05$). This might be due to similarity of the geographical nature of the areas from which the animals were sourced and sampled.

However prevalence rate varied significantly ($p < 0.05$) with body conditions in which higher prevalence was observed in poor body conditioned goats than that of medium and good body conditioned ones. This variation may be due to the possible associations of higher prevalence of brucellosis occurrence in the presence of various infectious diseases that can lead to the reduction of body weight, such as tuberculosis (Gorsich, 2012).

The prevalence of goat brucellosis in young animals in this study was 0.40% (1 out of 247), while that of adults was 1.97% (4 out of 203). In fact *B.melitensis* causes disease only in adult (sexually mature) females and males. Young animals may be infected but do not show any clinical sign and generally show only a weak and transient serological response (European Commission, 2001).

Statistical analysis of the data showed that there was no significant difference in sero-prevalence of *Brucella* antibodies between age groups, though older age group showed relatively higher prevalence. This finding was in agreement with previous reports of Tsehay *et al.* (2014) and Nigatu *et al.* (2014) who did not observe statistically significant difference between the sero-prevalence of brucellosis and age. Ashenafi *et al.* (2007) also reported a higher prevalence of brucellosis in adult goats in pastoral region of Afar, than younger ones which was in agreement with this study, however statistically significant relation was recorded between sero prevalence of brucellosis and age category ($p<0.05$). This difference might be due to variations in sample size and sample collection areas.

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6. CONCLUSION AND RECOMMENDATIONS

Brucellosis is one of the “neglected diseases of poverty” which is endemic zoonotic disease that is found primarily in impoverished parts of the world that are heavily reliant on livestock agriculture (World Health Organization, 2006; Blasco and Molina-Flores, 2011).

The sero prevalence study carried out in this study and the studies conducted so far in Ethiopia indicated that, Brucellosis might be one of the important diseases in goat raising districts.

In the present study, relatively low number of sero-reactors was identified in those export abattoirs. Even though no statically significant difference were recorded in the prevalence rates between the categories of each risk factors tested, there was a statistically highly significant difference on the prevalence rate of brucellosis among different categories of body conditions, where the disease was highly prevalent in poor conditioned goats than medium and good conditioned ones.

Though the causative agent, *Brucella* is not resistant to mild unfavorable environmental conditions and may die at lower pH of meat, positive sero-reactors existence in those animals subjected for slaughter may lead to ban of export of meat and meat-products to avoid zoonotic risks. Therefore it is strongly recommended to perform screening tests in goats that are supplied to abattoirs before slaughtering process, improve awareness of animal owners about the risk of brucellosis and its mode of transmission to control the dissemination of the disease.

Therefore based on the above conclusion the following recommendations are forwarded:

- Brucellosis positive animals can be potential hazard to susceptible animals and public health; therefore, screening test should be practiced before selection of animals for slaughter.
- Further and detail epidemiological study should be undertaken in those areas that supply livestock for export abattoirs to know the level and the trend of the disease dynamics, and to estimate the economic significance of the disease
- Even though the prevalence reported in this study was low, it can be potential hazard for public health in the study area; therefore, the public especially small ruminant producers should be aware of the risk of brucellosis.

- For both human and animal brucellosis, extension services should include emphasis on addressing the impacts of risk factors for the occurrence of brucellosis. Furthermore, interdisciplinary collaboration and joint ventures among health and related professionals is of paramount importance to control this disease that currently perpetuates poverty.

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8. ANNEXES

Annex 1: Estimation of Age of Goats

Number of Permanent teeth	Estimated age range of goats
None	Less than 1 years
1 pair	1 -2 years
2 pair	2-3 years
3 pair	3-4 year
4 pair	More than 4 year
Broken	Aged

Source: Desta (2009).

Annex 2: Data Collection Sheet

Date ……....

Region ……

Zone ……

District ……

Name of abattoir …….

Case No.	Risk factors				
	Origin	Species	Sex	Age	Body condition

Annex 3: Rose Bengal Plate Test (RBPT)

a. Materials

- White Porcelain Plate
- Micropipette of 75µl and 25 µl
- Micropipette tips
- Applicator sticks

b. Reagents

- RBPT Brucella antigen
- Positive control serum
- Negative control serum/ from previously tested negative serum/
- Test sera

c. Test Procedure

- The antigen, control and test sera were removed from the refrigerator and kept at room temperature for 30 minutes before the test commenced.
- White porcelain and glass plates with twelve shallow wells
- Then 75µl of sera were dispensed into each well using micro pipette in a direction from left to right as it was labeled on the plate (except well '3' and well '4' of raw '3' of the plate, which were dispensed with positive and negative control sera respectively, all other wells were dispensed with test sera).
- After the sera has been distributed, the antigen bottle was shaken then 25µl antigen was dispensed along side of each serum
- Then the antigen and the sera were mixed using applicator stick
- Timer was set for 4 min and plate was rocked manually backward and forward until 4 minutes elapsed
- After testing and reading the results plates were sent to washing and sterilization room where they were sterilized and wrapped in aluminum foils and made ready for re-use.

d. Interpretation of results

- After four minutes of rocking, any visible agglutination was considered as positive (OIE, 2009).
- Agglutination was recorded as 0, +, ++, and +++
- According to the degree of agglutination, a score of '0' indicates the absence of agglutination, '+' indicates barely perceptible agglutinations, '++' indicates fine agglutination, and '+++' indicates clear clumping
- Those samples with no agglutination, '0' were recorded as negative while those with '+', '++', and '+++' were recorded as positive

Annex 4: Complement Fixation Test

A. Reagents

- Complement
- Hemolytic serum (Amboceptor)
- Hemolytic system
- Brucella antigen
- Sheep Red Blood Cells (sRBC)
- Brucella abortus positive control serum
- Negative reference serum
- CFT buffer
- Alsever's solution

B. Materials

- 96 well U bottomed micro plate
- Mono-channel, Multi-channel micropipette which enable to dispense 25µl
- Water bath (NICKEL ELECTRO)
- Incubator shaker + 37^{o}C (INSL-England)
- Bench to centrifuge (SIGMA 4-10)
- Trough
- PH meter
- Syringe

- Sheet or plate lay out for records
- Shaker (R 100 rotates shaker)
- View box

C. Titration of the complement

1. First a 1/40 dilution of the complement in Veronal Calcium Magnesium (VCM) buffer was prepared. Then the following dilution in micro plates; all volumes were expressed in µl

2. Then the micro plates were dispensed in the quantities and orders shown in the table below

Well no	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
1/40 complement	10	15	20	25	30	35	40	45	50
VCM	40	35	30	25	20	15	10	5	0
Antigen	25	25	25	25	25	25	25	25	25

3. The mixture was homogenized by flipping the plates gently and put it at 37 ^{o}C for 30 minutes the we added

Hemolytic system	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**	**25**

4. The micro plate was incubated in incubator shaker at 37^{o}C for 30 minutes, then took out the micro plate well and centrifuged for 5 minutes at 2500 rpm.

5. The micro plate was removed from the centrifuge and the result was read. The quantity of complement in the first well to show complete hemolysis was unit 1 and the quantity in the next well was 2 units and used in the diagnostic test.

For example, if hemolysis is absent or incomplete in wells 1-4, 10 µl -25 µl and complete in wells 5 to 9, i.e. 30 µl-50 µl in this case 30 µl is 1 unit and 35 µl will be taken for diagnostic test which represent 2 units. Calculation takes the ratio of complement volume in the final dilution which 35 µl/100 µl= 0.35, using the formula 40/4×0.35=28. Then the dilution of the complement will be made 1/28 as indicated in the table below

Well dilution	1/100	1/66	1/50	1/40	1/33	1/28	1/25	1/22	1/20

D. Preparation of 3% sheep red blood suspension

- Blood was collected from Brucella antibody negative (upon CFT) sheep
- Blood was collected at a ratio of 50% blood to 50% Alsever's solution, mixed thoroughly by gentle rotation of the container
- Date of collection was recorded
- After collection, it was stored at +4^{o}C and 72 hours after collection it was ready for use

E. Hemolytic system

- It was prepared by mixing the Amboceptor working dilution 1:1 with the 3 % erythrocyte suspension then homogenized using R100 Rota test shaker for 30 minutes

F. Test Procedure

- Test sera, including positive and negative controls were Decomplemented in hot water bath at 58 ^{o}C for 30 minutes
- A U-shaped 96 microtiter plate wells was Prepared
- The test sera was diluted 1:2 (100 µl test serum in 100 µl VCM)
- We used column 1 through 12 for test samples
- We used each well of row 'A' for anti complementary control
- 25 µl of VCM was dispensed by using a hand-held 12 channel micro pipette into wells of rows A, C, D, E, F, and H

- 25 µl of diluted sera were dispensed in wells of rows A, B, C and we homogenized wells of rows C (column 1-12) of the test plate and picked up 25 µl from row C of the test plate and delivered to the wells of row D (column 1-12). This serial dilution was continued to row H (column 1-12) from which after homogenized 25 µl was picked up and discarded

    1. 25 µl of diluted antigen was added into all the wells of rows B, C, D, E, F, G, H and wells antigen and positive and negative control wells
    2. 25 µl of diluted complement was added in the wells of rows A, B, C, D, E, F, G, H and all control wells except complement and hemolytic system
    3. The plate was covered with sealer and incubated at +37^{o}C under constant agitation on incubator shaker for 30 minutes.
    4. 25 µl hemolytic system was added to all wells including control wells
    5. The plate covered with sealer and incubated at 37^{o}C under constant agitation on incubator shaker for 30 minutes

G. Control

- 25 µl of VCM was added into the wells of serum and antigen controls but for complement and hemolytic system 50 µl and 75 µl respectively
- 25 µl of control sera was added into the wells of first row at a dilution rate of 1/2 up to 1/8 for negative sera and 1/2 up to 1/128 for positive sera
- 25 µl of diluted antigen was added into all wells of antigen and from row B for the positive and negative controls
- **Complement control:** 25 µl of undiluted complement was added into the first well, and in the second well 25 µl of undiluted complement and 25 µl of VCM was added prior to homogenization. and transferred to third complement control well and 25 µl VCM into third and fourth well, then 25 µl of the mixture was homogenized and transferred into third and fourth well and finally 25 µl of the mixture from the fourth well.

E. Reading: the plate was put in a refrigerator at +4^{o}C for 1 hour to let non-hemolysed sRBC sediment, then the result was read over view box for degree of sedimentation or hemolysis.

Plate layout for test samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac	Ac
B	1/2											
C	1/4											
D	1/8											
E	1/16											
F	1/32											
G	1/64											
H	1/128											

For each set of reaction five controls must be included serum (positive and negative), Ag, C and HS.

Serum test at various two fold dilution 1:2, 1:4, 1:8, 1:16, 1:16, 1:32, 1:64, 1:128

- For serum controls first row A was used for anti complementary activity
- VCM was added to well A, C, D, E, F, G, and H
- serum was added 1:2 into well A, B and C then from C serially diluted and discarded (25 µl) from the last well for both positive and negative controls

F. Validation

i. Positive serum control: the inhibition of hemolysis should occur up to the expected dilution.
ii. Negative serum control: should show complete hemolysis
iii. Antigen control: complete hemolysis is expected
iv. Complement control: complete hemolysis in the first two wells (pure and 1/2), partial hemolysis in the third well (1/4) trace of hemolysis in fourth well (1/8)
v. Hemolytic system control: no hemolysis

Annex 5: Body Condition Scoring

Poor body condition: spinous process is prominent and sharp, the transverse processes are also sharp, the finger pass easily under the ends, and it is possible to feel between each process. The eye muscle areas are shallow with no fat cover.

Moderate body condition: the spinous process is detected only as a small elevation, it is smooth and rounded and individual bone can be felt only with pressure. The transverse process is smooth and well curved, and firm pressure is required to feel over the ends. The eye muscle area is full and has a moderate degree of the fat cover.

Good: the spinous process can just be detected with pressure as hard line between the fat covered eyes muscles area is full, and has thick covering of the fat

Source: Alemu and Merkel (2008).

9. DECLARATION

I, the under signed, declare that the information presented here in my thesis is my original work, has not been presented for degree in any other university and that all sources of materials used for the thesis have been duly acknowledged.

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Date of submission: JUNE 10, 2015

This thesis has been submitted for examination with my approval as university advisor

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